

AMENDMENTS TO THE SPECIFICATION

Please delete the entire Appendix 1 from the specification and replace the current sequence listing with a substitute sequence listing, submitted herewith.

Please delete the paragraph beginning at page 16, line 4:

~~Figure 6 shows a region of PspA which is highly conserved. A consensus sequence which is selected such that it conforms with formula I is also shown.~~

Please replace the paragraph beginning at page 16, line 7, with the following amended paragraph:

--Figure ~~[[7]]~~ 6 shows the CD spectrum of peptide CVX0270, ~~which contains a portion of the consensus sequence shown in Figure 6.~~--

Please replace the paragraph beginning at page 16, line 10, with the following amended paragraph:

--Figure ~~[[8]]~~ 7 shows the thermal denaturation profile of peptide CVX0270.--

Please replace the paragraph beginning at page 23, line 18, with the following amended paragraph:

--The PspA sequence from *S. pneumoniae* strain Rx1 was analyzed using the Peptools program from Biotools Inc. The Peptools structure prediction algorithm is based on identifying known protein-folding motifs combined with consensus prediction based on the results from four prediction algorithms. The Peptools program also predicts a highly helical region that spans over 300 amino acids for the N-terminal portion of PspA. Peptools program is also capable of predicting which sites of proteins are antigenic by measuring hydrophilicity, solvent accessibility, flexibility, and turn propensity of various sequences, and in addition, matches sequences against a database of known B-cell epitopes. ~~Peptools identified a number of potential B-cell epitopes in the coiled-coil domain which are listed in Appendix 1, which is incorporated herein by reference in its entirety.~~--

Please replace the paragraph beginning at page 42, line 4 and ending at page 42, line 15 with the following amended paragraph:

LEEAEKK ATEAKQK VDA 153-168 of SEQ ID NO:1

AELNQ VHRLEQE LKELDES 181-198 of SEQ ID NO:1

+

~~CN1eG IXXLXXX IXXLXXX IXXLXXX IXXLXXX IXXLXXX~~

coiled-coil template

[[ - ]]

~~CN1eG IEELEKK ITELKQK I - LENQ IHRLEQE IKELDES~~

[[ - ]]

↓

fill gap with aa from either sequence

CN1eG IEELEKK ITELKQK IDALENQ IHRLEQE IKELDES

(test peptide; SEQ ID NO:3)

Please replace the paragraph beginning at page 44, line 18, with the following amended paragraph:

--A consensus sequence (~~Figure 6~~) was deduced from a conserved region which was shown to have antigenic activities. Complete and partial PspA sequences were downloaded from the national Library of Medicine PubMed web site into the bioinformatics software Peptools (Biotools, Edmonton, Alberta). A total of 40 complete and partial sequences were transferred to the alignment module of the software and a consensus threshold was set to 65%. The consensus threshold defines the minimum residue plurality amongst a group of aligned sequences. In effect, the consensus threshold acts to filter out insignificant matches and highlights conserved residues within a sequence. This allows for easy identification of similarities by visual inspection.--

Please replace the paragraph beginning at page 45, line 1, with the following amended paragraph:

--The last 100 amino acids of the coiled-coil region were the focus of sequence similarities since this region has been shown previously to harbor cross reactive epitopes. ~~Figure 6 illustrates the~~ A sequence alignment for residues found within this region. ~~In particular, the alignment yielded~~ a consensus sequence of EELX<sub>1</sub>X<sub>2</sub>KIDELDX<sub>3</sub>EIAX<sub>4</sub>LEKX<sub>5</sub> (SEQ ID NO: 5), in which the putative a and d positions were selected to be isoleucine and leucine, respectively. Preferably, X<sub>1</sub> is S, Q, N or D; X<sub>2</sub> is D, N or K; X<sub>3</sub> is A or N; X<sub>4</sub> is K, E or D; and X<sub>5</sub> is N, D or E.--

Please replace the paragraphs beginning at page 45, line 24, and ending at page 46, line 21, with the following amended paragraphs:

--The CD spectra were performed under benign conditions (50 mM KH<sub>2</sub>P04, 100 mM KCl, pH 7.0) and in aqueous buffer containing 50% TFE. The CD spectrum of peptide CVX0270 under benign conditions at 20°C is shown in Figure ~~[[7]]~~ 6. The CD spectra is typical of a helical peptide with minima at 222 nm and 209 nm and high positive ellipticity below 200 nm. Typically, the molar ellipticity at 222 nm ( $[\theta]_{222}$ ) has been used to measure helical content in peptide. For peptide CVX0270 this corresponds to a value of -29800 which indicates that this peptide is predominantly  $\alpha$ -helical. Theoretically, the ( $[\theta]_{222}$ ) value for a peptide of 27 residues is -33900 (Chen et al., 1974). Therefore peptide CVX0270 is 88% helical. It should be noted that the linker region (Cys-Nle-Gly) is designed not to be helical and therefore decreases the ( $[\theta]_{222}$ ) signal. The ratio of the molar ellipticity at 222 and 208 ( $[\theta]_{222}/[\theta]_{208}$ ) is greater than 1.02 and similar to that observed before for coiled-coils (Hodges et al., 1988; Lau et al, 1984; Zhou et al., 1992) and distinctly different from non interacting  $\alpha$ -helices in which the ( $[\theta]_{208}$ ) is greater than the ( $[\theta]_{222}$ ). In the presence of 50% TFE, helical content increased slightly to 101%. The data indicate that peptide CVX0270 is highly helical and found predominantly in the coiled-coil conformation under aqueous conditions.

In order to determine the stability of CVX0270, a thermal denaturation study was undertaken. Figure ~~[[8]]~~ 7 shows the denaturation curve obtained by monitoring the ( $[\theta]_{222}$ ) as a function of

temperature. The study indicates that peptide CVX0270 is very stable with the peptide exhibiting 77% of its original helicity at 75°C. The above results demonstrate that the coiled-coil forming sequence with isoleucine at a positions and leucine residues at d positions is sufficiently stable to house a helical epitope from another protein sequence.--